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Subchronic Inhalation and Oral Toxicity of *N*-Vinylpyrrolidone-2. Studies in Rodents

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Abstract—*N*-Vinylpyrrolidone-2 (NVP) is a monomeric compound used as an industrial intermediate. Nine of 11 studies previously reported involved exposure of rats (two different strains), mice or hamsters to NVP by the inhalation route at concentrations of up to 120 ppm (6 hr/day, 5 days/wk) over a period of 1 wk to 12 months. The remaining two studies involved exposure of rats to NVP through the drinking water or by gavage at dose levels of up to 100 mg/kg body weight/day. Reduced body weight gain was seen in rats exposed by inhalation to 5 ppm or more for 3 months and in mice and hamsters exposed to 45 ppm for only 1 day. Effects were seen on haematological (reduced haemoglobin, erythrocyte count, haematocrit) and clinical chemistry parameters (specially raised γ-glutamyltransferase activity and decreases in plasma protein), liver weight increase and liver lesions (centrilobular single-cell necrosis and foci of hepatocellular alteration) in rats and mice but not hamsters. Rats exposed to 40 mg/kg body weight/day NVP or more for 3 months by gavage developed similar liver changes. Atrophy of olfactory epithelium and hyperplasia of nasal respiratory epithelium was seen in rats exposed by inhalation to 5 ppm NVP for 7 wk but not in response to 1 ppm for 13 wk (no observed-adverse-effect level, NOAEL). These studies indicated that the upper respiratory tract and the liver are the main targets for NVP toxicity.

Abbreviations: AP = alkaline phosphatase; GSH = glutathione; γ-GT = γ-glutamyltransferase; NOAEL = no observed-adverse-effect level; NVP = *N*-vinypyrrolidone-2.

INTRODUCTION

N-Vinylpyrrolidone-2 (NVP), CAS registry no. 88-12-0 (synonyms: 1-vinyl-2-pyrrolidone, 1-ethenyl-2-pyrrolidone, vinylpyrrolidone) [boiling point 193°C (at 400 mmHg), freezing point 13.5°C] is an industrial raw material, used as a monomer in the production of homopolymers such as water-soluble and insoluble forms of polyvinylpyrrolidone, or as co-monomer, together with other suitable monomers, in the manufacture of polymers, mainly for the pharmaceuticals and cosmetics industry. It is also used as a reactive diluent in a variety of radiation curable coatings, inks and adhesives.

The possibility of exposure of chemical process workers is limited by the fact that high standards of occupational hygiene in industry operate where it is made and used. The only possibility of exposure of the general public relates to the emission of NVP from residual monomeric NVP in polymers.

Prior to, or in parallel with the studies reported in the present paper, other studies have concerned the toxicokinetics of NVP, its possible genotoxicity and the identification of targets for its toxicity. Most of these studies have not been published in the open literature; however, reference is made to them in the MAK Documentation (Greim, 1994) and the EUCLID Data Sheet (VCI, 1992). Thus, a study of the toxicokinetics of NVP in rats showed that iv-injected ^{14}C-labelled NVP was cleared from the blood with a half-life of about 2 hr; between 75 and 86% of the radioactivity was recovered from the urine within 12 hr of dosing; unchanged NVP accounted for less than 0.6% of the dose administered in this study, but the main metabolites were not identified. Several *in vitro* and *in vivo* tests of NVP for mutagenicity and clastogenicity have given negative results for genotoxicity, and no binding to DNA of the liver was found in rats following an ip injection of NVP.

Prior to the tests reported in the present paper, three target sites for NVP toxicity had been identified. First, NVP was known to be irritant to the skin and mucous membranes. Secondly, it was known to be hepatotoxic. Thirdly, it was known to

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Table 1. Design of studies involving exposure of mice, rats and hamsters to NVP by various routes

Study	Species	Exposure		Concentration in ppm	Animal number and sex per group	Scope of examinations*
		Route	Duration			
1	F344 rats	Inhalation	1 wk	0, 45	6 (M)	In plasma: total protein, albumin, GOT, GPT, AP; in liver homogenate: γ-GT, GSH, necropsy, liver histopathology.
2	Mice	Inhalation	1 wk	0, 45	6 (F)	In plasma: total protein, albumin, GOT, GPT, AP; in liver homogenate: γ-GT, GSH, necropsy, liver histopathology.
3	F344 rats	Inhalation	7 wk (sat. gr. 1 wk and 3 wk)	0, 5, 15, 45	20 (M + F)	Scope based on OECD method 412 including γ-GT in liver homogenate.
4	Mice	Inhalation	7 wk (sat. gr. 1 wk and 3 wk)	0, 5, 15, 45	20 (M + F)	Scope based on OECD method 412 including γ-GT in liver homogenate.
5	Hamsters	Inhalation	3 months (sat. gr. for 7 wk)	0, 45	40 (M + F)	In plasma: γ-GT; in liver homogenate: γ-GT, GSH; necropsy, histopathology: liver.
6	Sprague–Dawley rats	Inhalation	3 months	0, 1, 5, 15, 45, 120	10 (M + F)	Scope based on OECD method 413 including urinalysis and electrophoresis.
7	Wistar rats	Drinking water	3 months (sat. gr. for 3 + 4 weeks)	0, 5, 12, 30, 75 mg/kg body weight on 7 days/wk	10 (M + F)	Scope based on OECD method 408, additonally, γ-GT in liver homogenate.
8	Wistar rats	Gavage	3 months	0, 40, 60, 100 mg/kg body weight on 5 days/wk	5 (M + F)	Scope based on OECD method 407 including elecrophoresis; histopathology: liver; additionally: γ-GT in liver homogenate.
9	F344 rats	Inhalation	6 months	0, 10	10 (M + F)	Scope based on OECD method 413; histopathology: liver; additionally: γ-GT, GSH in liver homogenate.
10	Mice	Inhalation	6 months	0, 10	10 (M + F)	Scope based on OECD method 413; no histopathology; additionally GSH in liver homogenate.
11	Sprague–Dawley rats	Inhalation	12 months (sat. gr. 3 months)	0, 5, 10, 20	10 (M + F) sat.gr. 3 months: 20 (M + F) NVP groups	Scope based on OECD method 413; histopathology: liver, pancreas, nasal cavity; additionally γ-GT and GSH in liver homogenate; urinalysis.

F = females M = males sat. gr. = satellite group p.t. = post-exposure observation period

*Body weight determination and observation signs were always performed.

Table 2. Effects of inhalation exposure of male and female Sprague–Dawley rats to NVP for 3 months on body weight gain (study 11)

Group (ppm)		Day 1	Day 6	Day 13	Day 20	Day 27	Day 34	Day 41	Day 48	Day 55	Day 62	Day 69	Day 76	Day 83	Day 90
								Male							
0	M	282.2	324.2	358.0	389.0	414.1	432.9	452.7	464.8	476.3	490.9	505.3	509.7	527.4	527.0
	SD	1.7	24.7	28.9	30.8	37.1	42.2	44.3	40.8	52.9	52.9	50.9	47.7	54.8	56.6
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
5	M	275.2	315.9	344.2	378.5	397.8	406.2	429.5	447.3	462.1	476.6	492.7	502.2	510.2	521.3
	SD	28.5	33.2	38.2	43.8	45.6	48.2	49.9	52.1	52.7	54.8	54.7	55.8	57.9	61.2
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20
10	M	268.1	305.7	337.5	363.5	386.1	406.5	432.9	451.3	460.3	471.7	484.3	491.1	502.4	502.0
	SD	22.3	24.4	28.6	34.0	37.3	39.2	43.2	45.2	48.0	48.4	49.6	51.3	53.4	53.7
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20
20	M	265.6	289.9**	312.8**	338.9**	355.9**	374.1**	393.8**	405.8**	410.1**	426.2**	437.3**	444.2**	455.9**	460.0**
	SD	18.8	21.5	26.5	25.2	24.8	24.3	25.2	26.4	29.6	28.7	28.2	29.0	29.3	31.1
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20
								Female							
0	M	204.5	220.6	234.0	249.1	260.8	267.4	270.0	282.5	286.5	291.1	293.9	300.9	307.0	304.6
	SD	8.4	10.2	10.9	9.6	10.5	14.8	14.0	12.8	15.5	16.9	18.4	17.9	17.9	19.2
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
5	M	197.9	211.5	226.4	238.9	249.9	257.8	264.6	270.9	275.7	283.0	290.3	291.2	293.4	296.2
	SD	16.6	22.0	20.1	20.5	20.5	23.7	24.2	25.7	25.1	26.7	30.4	30.4	30.8	31.1
	N	20	20	19	19	19	19	19	19	19	19	19	19	19	19
10	M	200.6	215.5	234.2	246.8	259.2	264.3	275.8	281.8	283.1	286.8	291.9	299.4	305.0	301.0
	SD	14.7	16.3	18.4	18.2	18.8	20.4	22.6	22.4	22.5	25.0	24.9	26.3	25.9	27.7
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20
20	M	201.3	211.6	225.4	240.6	250.9	258.8	268.4	272.2	277.4	280.2	290.2	292.4	296.5	296.1
	SD	21.6	24.0	28.8	34.4	30.5	31.5	35.4	36.2	35.2	37.5	44.0	41.6	43.2	42.0
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20

Statistics: ANOVA and Dunnett's test; *$P < 0.05$; **$P < 0.01$ two-sided (statistical unit = animal).

Table 3. Effects of inhalation exposure of male and female Sprague–Dawley rats to NVP for 3 months on body weight gain (2-yr inhalation study; Klimisch *et al.*, 1997)

Group (ppm)		Day 1	Day 6	Day 13	Day 20	Day 27	Day 34	Day 41	Day 48	Day 55	Day 62	Day 69	Day 76	Day 83	Day 90
								Male							
0	M	282.6	320.9	355.3	380.6	401.7	420.0	436.3	456.5	468.4	483.6	491.5	505.7	511,5	522.4
	SD	24.6	28.5	29.9	34.6	37.3	38.6	40.8	43.8	46.1	47.6	493	51.5	53.1	55.1
	N	70	70	70	70	70	70	70	70	70	70	70	70	70	70
5	M	274.3	311.1	338.6**	366.2*	390.1	399.1*	424.1	439.0*	447.6*	456.5**	471.7*	480.2**	485.7**	491.9**
	SD	25.0	28.1	32.6	31.6	34.1	63.1	38.6	40.8	44.1	43.1	44.8	45.9	46.5	46.9
	N	60	60	60	60	60	60	60	60	60	60	60	60	6	60
10	M	268.1**	304.0**	335.6**	359.5**	381.3*	399.0*	420.7	433.3**	444.7**	455.1**	4683.4*	481.6*	487.9*	488.5**
	SD	21.2	23.4	26.5	29.5	31.1	31.1	37.3	38.3	40.7	40.4	42.0	44.8	45.9	46.6
	N	60	60	60	59	59	59	59	59	59	59	59	59	59	59
20	M	275.4	297.2**	325.3**	351.3**	370.3**	381.5**	402.8**	412.2**	422.6**	433.7**	444.4**	452.0**	461.8**	464.5**
	SD	24.1	26.1	30.3	33.6	36.0	39.5	44.6	44.6	48.5	46.7	49.6	50.1	51.3	51.2
	N	60	60	60	60	60	60	60	60	60	60	60	60	60	60
								Female							
0	M	203.6	218.8	236.1	249.7	259.4	268.6	272.6	281.4	287.8	292.6	299.0	304.3	306.8	311.2
	SD	15.3	17.4	19.8	20.7	21.3	22.8	25.9	25.9	26.8	29.1	30.8	30.9	31.2	30.6
	N	70	70	70	70	70	70	70	70	70	70	70	70	70	70
5	M	200.43	216.2	229.3	239.66**	253.0	258.3	265.2	272.7	281.3	283.9	291.1	294.8	297.7	300.6
	SD	16.0	18.4	18.1	20.4	21.9	21.6	21.9	24.5	25.3	26.1	26.9	30.6	28.8	29.9
	N	60	60	60	60	60	60	60	60	60	60	60	60	60	60
10	M	200.6	214.6	228.1*	240.5*	249.1*	257.6*	265.1	272.7	276.0*	280.0*	286.4*	293.2	293.3*	294.0**
	SD	14.9	16.2	17.5	18.4	19.8	21.2	21.6	23.3	23.3	24.2	24.7	26.1	27.2	30.0
	N	60	60	60	60	60	60	60	60	60	60	60	60	60	60
20	M	199.2	208.9**	226.9*	237.6**	247.9**	258.8*	263.0	267.7**	271.7**	276.6**	283.5**	288.5**	289.4**	295.6**
	SD	15.4	16.0	18.6	18.3	18.8	20.1	20.6	20.3	22.9	22.3	21.6	23.4	25.1	27.0
	N	60	60	60	60	60	60	60	60	60	60	60	60	60	60

affect certain peripheral blood parameters (Greim, 1994; VCI, 1992).

Nine of the 11 studies described in the present paper (see Table 1) were aimed at defining the toxicity profile of NVP under conditions of repeated exposure by the inhalation route, this being the route of exposure most relevant to occupational safety. The two remaining studies (Nos 7 and 8) involve repeated exposure by the oral route. A further aim of the nine inhalation studies was to provide guidance for the selection of exposure concentrations suitable for use in a long-term toxicity/carcinogenicity study in rats (Klimisch *et al.*, 1997).

For comparison with the body weight data derived from study 11 (see Table 2) data derived from the first phase of a long-term inhalation study are shown in Table 3.

MATERIAL AND METHODS

Test chemical

The purity of NVP used in the present studies was 99.9%. Previous experience established that NVP is stable for 9 months when stored at temperatures of between 5 and 20°C. Therefore the compound was stored at 15 ± 2°C.

Design of studies

Studies of different duration (ranging from 1 wk to 12 months) were performed in three different species (rats, mice, hamsters), by two different exposure routes (inhalation, oral) and with the use of various measurements of response. In the case of experiments 3, 4, 5, 7 and 11 satellite groups of animals were included in the study design. These groups were terminated earlier than the main groups. An overview of the study designs is shown in Table 1. In general, studies were performed in compliance with GLP requirements between March 1983 and the end of 1987.

Similar experimental procedures were used in the various studies in relation to the maintenance and observation of the animals, the generation of NVP vapour, the inhalation exposure system, the analytical methods used to measure NVP vapour concentrations, the haematological and clinico-chemical determination procedures, urinalysis and necropsy and histopathological evaluation. Thus, the details given in the following paragraphs apply, as appropriate, to all the studies.

Animals and maintenance. Specific pathogen-free (SPF) male and female Sprague–Dawley rats (Crl:CD(SD)Br, 37–39 days old), SPF male and female Fischer 344 rats (CDF9F-344)-CrlBr, SPF male and female C57BL/6NCrl, Br mice were purchased from CIVO–TNO (Zeist, The Netherlands). Male and female Syrian golden hamsters (Cpb-ShGa51) were obtained from TNO (Zeist). Rats were identified individually by an ear-tattoo number (Klimisch, 1986). Except when they were being exposed to NVP, rats were housed in pairs in stainless-steel wire-mesh cages, mice were housed singly in stainless-steel wire-mesh cages and hamsters were housed singly in Makrolon® cages with type M bedding (Becker, Castrop-Rauxel). The animals were randomly assigned to the test groups (Table 1) using a computer-based randomization plan. The different concentrations/doses are shown in Table 1. The animals were maintained in air-conditioned rooms at 22 ± 2°C with a relative humidity of 55 ± 10% and a 12 hr light/dark cycle (light between 06.00 and 18.00 hr).

Food: Maintenance pelleted laboratory diet, obtained from Kliba 23-343-4 (Klingentalmühle AG, Kaiseraugst, Switzerland) and drinking water (tap water of the municipality of Frankenthal, analysed according to BGBl recommendations and meeting BGBl purity standards, 1986) were provided *ad lib.* between inhalation exposure periods but were withdrawn from animals during exposure. Each batch of diet was assayed for bacterial and chemical contaminants (according to EPA Guidelines, 1979). The drinking water was similarly assayed at regular intervals. The results of these analyses provided no evidence of contamination that might have compromised the study.

Inhalation system. One module of a horizontal flow stainless-steel inhalation chamber as described in detail elsewhere (Klimisch *et al.*, 1997) with a chamber volume of 4.2 m^3 was used for each exposure group. The inhalation chambers were installed in a room maintained as described above. Animals were exposed 6 hr/day on 5 days/wk.

Generation and analysis of NVP inhalation atmospheres. NVP was evaporated under mild conditions by generating a fine-particle aerosol and evaporating these particles at temperatures lower than 55°C. NVP vapour concentrations in the inhalation chambers were monitored four to six times during each 6-hr exposure period using a calibrated total hydrocarbon analyser (THA Ratfisch 5S 55, München, Germany).

Calibration was carried out using a gas-chromatographic method which was also used to validate NVP concentrations in the inhalation chambers at least once each week. Concentration deviations never differed by more than 5% from the target concentrations. Further details of the method used for the generation and analysis and the methods used for monitoring inhalation chamber conditions (temperature, relative humidity, pressure and air flows) are published elsewhere (Klimisch *et al.*, 1997).

Exposure through the drinking water. NVP solutions in the drinking water at concentrations of 5, 12, 30 and 75 ppm were prepared twice weekly (NVP stability for at least 4 days had been checked previously by analysis). The concentrations of NVP in the drinking water were determined at the start of the study, after 6 wk and at the end of the study (the means found were 5, 11.5, 28.7 and 76.3 ppm, respectively). The NVP/drinking water preparations were dispensed into the drinking bottles using a

semi-automatic metering device (FORTUNA-OPTI-FIX, Germany). Water consumption was determined twice weekly and the total dose (ml/kg body weight/day) was calculated.

Gavage study. The animals were exposed by gavage once daily on 5 days/wk, to 5 ml aqueous NVP/kg in concentrations designed to provide 40, 60 or 100 mg/kg body weight/day). The solutions were freshly prepared daily. Control animals received 5 ml/kg body weight/day water only by gavage. The concentrations of the NVP solutions were adjusted so that the animals always received the same volume by gavage (5 ml/kg body weight) at each administration. The concentrations of the administered solutions were analytically checked at the start of the study, after 4 and 12 wk.

Body weight measurements. Except in the 1-wk studies, in which animals were weighed on alternate days, animals were weighed once weekly during the first 3 months and thereafter once monthly.

General health, clinical signs and behaviour. Animals were observed through the glass windows of the exposure chambers twice daily during exposure periods. A check was also made for moribund or dead animals before and after each exposure period. In addition each animal was physically examined once weekly.

Ophthalmoscopy. Ophthalmological examinations for changes in the refracting media were performed according to the OECD guidelines (OECD, 1981) using a focusable Heine–Focalux hand-held slit lamp before the first exposure and shortly before the termination of the study.

Haematology. Haematological examinations were carried out generally at various times during studies and in most cases prior to the termination of studies. Individual blood samples from different species were obtained by bleeding through the retro-orbital sinus from non-fasted animals 1 day after exposure. Haematological measurements were made on blood samples containing EDTA as an anticoagulant using a Coulter Counter S plus analyzer (Coulter Electronics Inc., Hialeah, FL, USA). Each blood sample was analysed for the following parameters: red blood cell count, haemoglobin, mean corpuscular volume, platelet count and white blood cell count. Erythrocyte indices (mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration) and haematocrit were calculated. Differential white blood cell counts were evaluated microscopically or by an automatic differential system (Hematrak 480, Geometric Data, Wayne, PA, USA). Reticulocytes were counted in the 12-month inhalation study using a Hematrak analyzer. For clotting analyses, prothrombin time was measured with a ball coagulometer KC 10 (Amelung, Lemgo, Germany).

Table 4. Body weight decrease after 3-month inhalation of NVP in a long-term rat study compared with the control group (see study 2 and Klimisch *et al.*, 1997)

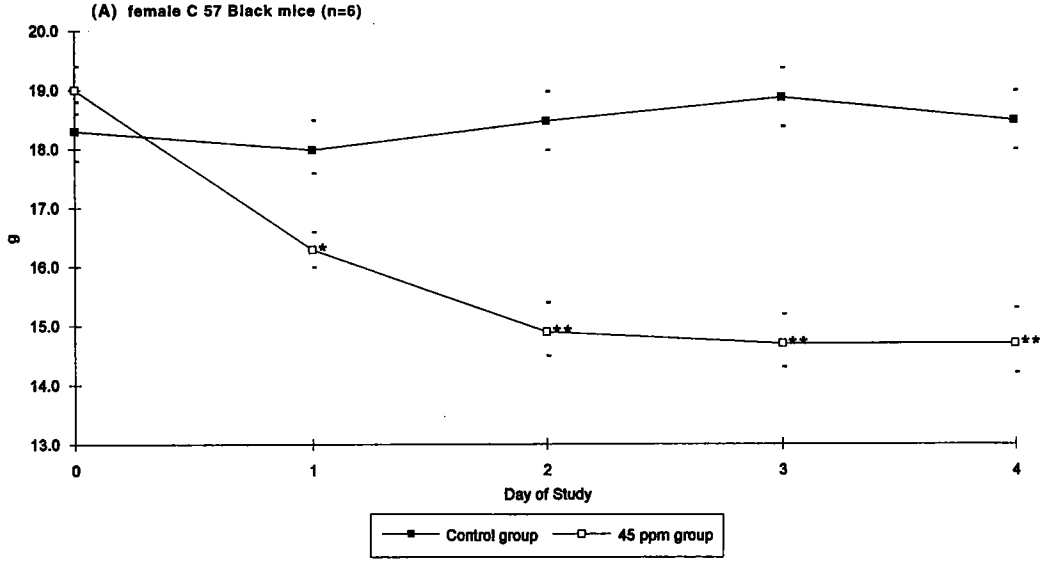
	Decrease (%) after 3 months	
	Females (n = 60)	Males (n = 60)
5 ppm	3.4	5.9
10 ppm	5.6	6.5
20 ppm	5.0	11

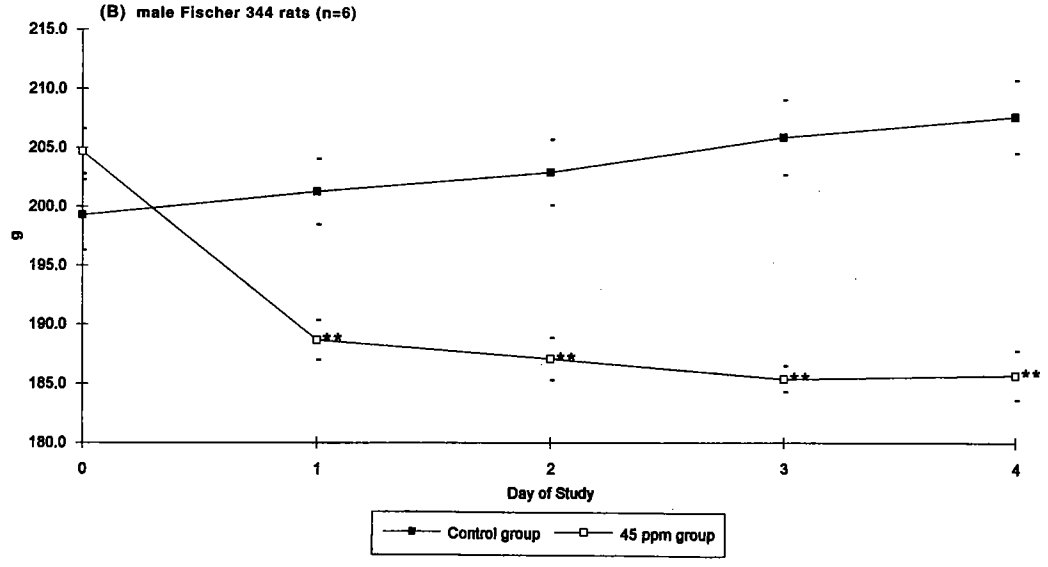
Clinical chemistry. Clinical chemistry examinations were performed at various time intervals throughout studies and at the end of most studies. Blood was collected from the retro-orbital sinus. The following parameters were measured in plasma containing heparin as an anticoagulant using a Greiner Selective Analyzer II (Greiner, Langenthal, Switzerland): sodium, potassium, chloride, total bilirubin, creatinine, urea, total protein, albumin, glucose, inorganic phosphate, calcium, triglycerides and cholesterol. Enzyme activities were determined with an enzyme analyzer ACP 5040 (Eppendorf, Hamburg, Germany). In most studies the following enzymes were measured in the plasma: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase (AP), lactate dehydrogenase, γ-glutamyltransferase (γ-GT) and cholinesterase. In some studies, γ-GT activity and glutathione (GSH) concentration were determined spectrophotometrically in liver homogenates and plasma protein levels were measured by electrophoresis (see Table 1).

Urinalysis. Urine was collected for routine analysis at various times in two studies (see Table 1). For urine collection rats were placed overnight in individual metabolism cages and urine was collected over a period of 16 hr. The animals had access to water but not to food. The following morning urine samples were analysed for nitrite, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood and specific gravity. Chemical analyses were performed using reagent strips 'Combur-9-test RL' and an Urotron RL 9 reflection photometer (Boehringer, Mannheim, Germany). Specific gravity was measured with a refractometer and urine sediments were examined microscopically.

Gross pathology, histopathology. Animals were killed by exsanguination from the abdominal aorta and vena cava under Narcoren® anaesthesia. They were necropsied and assessed for gross pathology. Liver, kidneys, adrenal glands, lungs, brain and testes were weighed. The following organs and/or tissues were fixed in 4% formole saline solution: brain, pituitary gland, thyroid with parathyroid glands, thymus, lungs, larynx, trachea, heart, salivary glands, spleen, kidneys, adrenal glands, tongue, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, uterus, urinary bladder, lymph nodes, pancreas, testes/ovaries, acces-

Fig. 1. Body weight changes after inhalation exposure to 45 ppm NVP for 6 hr per day over different exposure periods [with standard error of mean x = SEM and significance levels ($^*P > 0.05$; $^{**}P > 0.01$)]. A = female C57Bl mice (study 2); B = male F344 rats (study 1); C = male Syrian golden hamsters (study 5).


(A) female C 57 Black mice (n=6)
20.0
19.0
18.0
17.0
16.0
15.0
14.0
13.0
g
0
1
2
3
4
Day of Study
Control group
45 ppm group


(B) male Fischer 344 rats (n=6)
215.0
210.0
205.0
200.0
195.0
190.0
185.0
180.0
g
0
1
2
3
4
Day of Study
Control group
45 ppm group

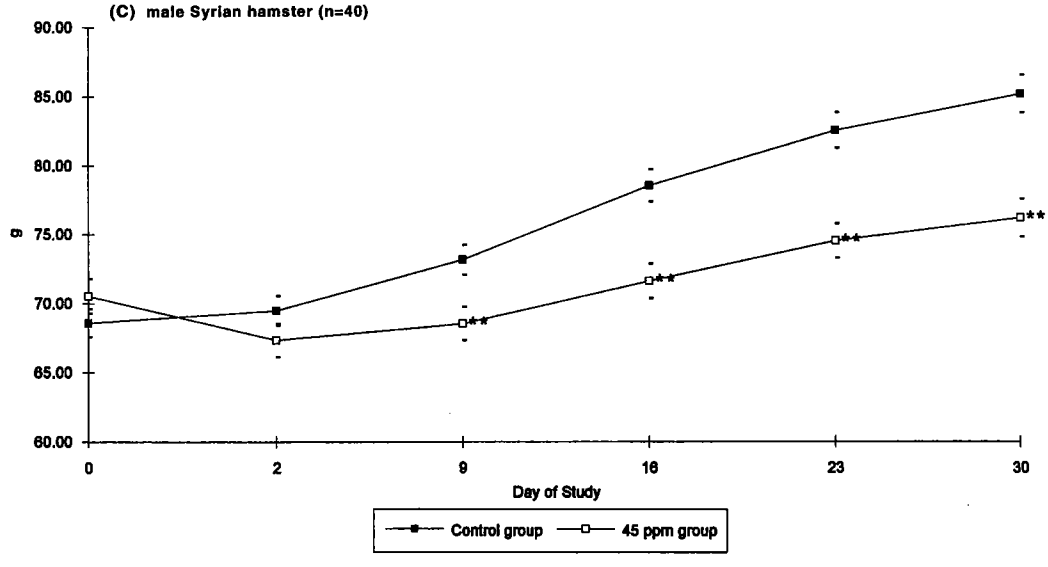

(C) male Syrian hamster (n=40)
90.00
85.00
80.00
75.00
70.00
65.00
60.00
g
0
2
9
16
23
30
Day of Study
Control group
45 ppm group

Fig. 1(a)–(c).

Table 5. Treatment-related differences between NVP-exposed and control animals in studies 3–11* (with the exception of the differential blood count all changes are statistically significant at least at the $P < 0.05$ level)

Study	Type of study	Species/doses	Results	
			Clinical chemistry	Haematology
3	Inhalation 1, 3, 7 wk	F 344 rats 5, 15, 45 ppm	*Decrease in total protein*: 15 ppm M (1 wk), F (1, 3 wk); 45 ppm M + F (1, 3 wk) *Decrease in albumin*: 15 ppm M (1 wk); 45 ppm M (1, 3 wk) F (7 wk) *Decrease in globulins*: 15 ppm M (1 wk), F (1, 3 wk; 45 ppm M (1, 3, 7 wk), F (1, 3 wk) *Increase in triglycerides*: 15 ppm M (3 wk); 45 ppm M + F (1, 3 wk) *Increase in cholesterol*: 15 ppm M + F (7 wk); 45 ppm M (1, 7 wk), F (7 wk) *Increase in total bilirubin*: 15 ppm M (7 wk); 45 ppm M (1, 3, 7 wk), F (3 wk) *Increase in alanine aminotransferase*: 45 ppm M + F (1, 3 wk) *Increase in aspartate aminotransferase*: 45 ppm M + F (1 wk) *Increase in alkaline phosphatase*: 45 ppm M (3 wk), F (1, 3 wk) *Increase in liver γ-glutamyltransferase*: 15 ppm F (7 wk); 45 ppm M + F (1, 3, 7 wk) *Increase in liver glutathione-SH*: 15 ppm M (1, 3 wk), F (3, 7 wk); 45 ppm (1, 3, 7 wk), F (3, 7 wk) *Decrease in creatinine*: 45 ppm M (7 wk)	*Increase in haemoglobin*: 45 ppm M (1 wk), F (1, 3 wk) *Decrease in haemoglobin*: 15, 45 ppm M + F (7 wk) *Increase in erythrocytes*: 45 ppm (1 wk), F (1, 3 wk) *Decrease in erythrocytes*: 15, 45 ppm M + F (7 wk) *Increase in haematocrit*: 15, 45 ppm M + F (7 wk) *Decrease in haematocrit*: 15, 45 ppm M + F (7 wk) *Decrease in mean cell volume*: 15, 45 ppm F (7 wk) *Increase in mean corpuscular haemoglobin*: 15, 45 ppm F (7 wk) *Increase in platelets*: 15 ppm M (7 wk), 45 ppm M (3, 7 wk), F (7 wk) *Increase in polychromasia*: 45 ppm M (7 wk)
4	Inhalation 1, 3, 7 wk	$C57B_1$ mice 5, 15, 45 ppm	*Decrease in total protein*: 15 ppm M (3, 7 wk); 45 ppm M + F (3, 7 wk) *Decrease in albumin*: 15 ppm M (3, 7 wk), F (3 wk); 45 ppm M + F (3, 7 wk) *Decrease in globulins*: 15 ppm M (3 wk); 45 ppm M + F (3 wk) *Increase in liver glutathione-SH*: 15 ppm M (1 wk), F (3 wk); 45 ppm M + F(1, 3, 7 wk)	*Decrease in haemoglobin*: 15, 45 ppm M (7 wk) *Decrease in erythrocytes*: 15, 45 ppm M (7 wk) *Decrease in haematocrit*: 15, 45 ppm M (7 wk) *Decrease in mean cell volume*: 45 ppm M (1, 3, 7 wk), F (3 wk) *Increase in platelets*: 15 ppm (7 wk), F (3, 7 wk) *Increase in neutrophils*: 5, 15, 45 ppm M (1 wk) *Increase in lymphocytes*: 45 ppm M (7 wk)
5	Inhalation 3 months	Syrian hamsters, 45 ppm	No changes	No histopathology performed
6	Inhalation 6, 13 wk	Sprague–Dawley rats 1, 5, 15, 45 ppm	*Increase in total bilirubin*: 15 ppm M (6 wk); 45 ppm M + F (6, 13 wk) *Decrease in total protein*: 5 ppm (6,13 wk); 15 ppm M + F (6 wk); 45 ppm M + F (6, 13 wk) *Decrease in albumin*: 5 ppm F (6,13 wk); 15 ppm M + F (6 wk); 45 ppm M (13 wk), F (6, 13 wk) *Decrease in globulins*: 5 ppm M + F (13 wk); 15 ppm M + F (6 wk) 45 ppm M (6,13 wk), F (6 wk) *Decrease in α_1-globulins*: 5 ppm F(13 wk); 15 ppm M + F (13 wk); 45 ppm M + F (13 wk) *Decrease in cholesterol*: 45 ppm M (6,13 wk); F (6 wk) *Decrease in alkaline phosphatase*: 15 ppm M (13 wk); 45 ppm M (6, 13 wk) *Decrease in creatinine*: 5, 15, 45 ppm F (6 wk) *Decrease in urea*: 5 ppm F (6 wk); 15, 45 ppm M + F (6 wk) *Decrease in potassium*: 5 ppm F (6 wk); 45 ppm M (13 wk), F (6, 13 wk)	*Decrease in haemoglobin*: 15 ppm M (6 wks), F (6,13 wk); 45 ppm M (6 wk), F (6,13 wk) *Decrease in haematocrit*: 15 ppm F (6,13 wk); 45 ppm M (13 wk), F (6,13 wk) *Decrease in mean corpuscular haemoglobin*: 45 ppm M (6 wk), F (6,13 wk) *Decrease in mean corpuscular volume*: 15 ppm F (13 wk); 45 ppm M + F (6,13 wk) *Increase in platelets*: 15 ppm M (6,13 wk); 45 ppm M + F (6, 13 wk) *Increase in neutrophils*: 15 ppm M + F (6,13 wk); 45 ppm M + F (6,13 wk) *Increase in lymphocytes*: 15 ppm M (6,13 wk); 45 ppm M + F (6, 13 wk)

7	Drinking water 3 months	Wistar rats, 0.5, 1.2, 3.0, 7.5 mg/kg/day (7 days per wk)	*Decrease in total protein*: 7.5 mgkg M + F *Decrease in albumin*: 7.5 mg/kg F *Decrease in globulins*: 7.5 mg/kg M + F	No changes
8	Gavage 3 months	Wistar rats 40, 60, 100 mg/kg (5 days per wk)	*Increase in liver γ-glutamyltransferase*: 40, 60, 100 mg/kg M + F	*Increase in platelets*: 60, 100 mg/kg M + F
9	Inhalation 6 months	F344 rats 10 ppm	*Increase in liver γ-glutamyltransferase*: M + F *Increase in liver glutathione-SH*: F *Decrease in total protein*: F *Decrease in albumin*: F *Decrease in globulins*: M *Decrease in alanine aminotransferase*: M + F	*Decrease in haematocrit*: M + F *Decrease in mean corpuscular volume*: M + F *Increase in platelets*: M + F
10	Inhalataion 6 months	C57 Bl mice 10 ppm	*Increase in liver glutathione-SH*: M + F *Decrease in total protein*: M + F *Decrease in globulins*: M + F	No histopathology performed
11	Inhalation 3, 12 months	Sprague–Dawley rat 5, 10, 20 ppm	*Decrease in total protein*: 5 ppm M + F (3 months); 10 ppm M (3 months), F (3, 12 months); 20 ppm M (3 months), F (3, 12 months) *Decrease in albumin*: 5 ppm F (3 months); 10 ppm F (3, 12 months); 20 ppm F (3, 12 months) *Decrease in globulins*: 5 ppm M + F (3 months); 10 ppm M (3 months), F (3, 12 months); 20 ppm M + F (3 months) *Increase in cholesterol*: 20 ppm F (12 months) *Increase in γ-glutamyltransferase*: 20 ppm M (12 months), F (3,12 months) *Increase in liver-glutathione-SH*: 10 ppm M (3 months); 20 ppm M + F (3, 12 months) *Increase in neutrophils*: 20 ppm F (12 months) *Increase in lymphocytes*: 20 ppm F (12 months) *Increase in monocytes*: 20 pm F (12 months) *Increase in reticulocytes*: 20 ppm F (12 months) *Increase in micorcytosis*: 20 ppm F (12 months) *Increase in anisocytosis*: 20 ppm M + F (12 months)	*Decrease in haemoglobin*: 20 ppm F (12 months) *Decrease in haematocrit*: 20 ppm F (12 months) *Decrease in mean corpuscular volume*: 20 ppm F (12 months) *Decrease in mean corpuscular haemoglobin*: 20 ppm F (12 months) *Decrease in mean corpuscular haemoglobin concentration*: 20 ppm F (12 months) *Increase in platelets*: 10, 20 ppm M + F (3 months)

F = Female M = Male

*No measurements were made in studies 1 and 2.

sory genital organs, female mammary gland, skin, sciatic nerve with attached skeletal muscle, spinal cord, sternum, femur, eyes, and all gross lesions. Sections of liver were also fixed in (a) Carnoy's solution, and (b) acetic acid–ethanol solution.

The liver, nasal cavity (four levels), pancreas and gross lesions were embedded in paraffin and stained with haematoxylin and eosin. Further sections of the liver were stained for glycogen, γ-GT and fat. Microscopic examination was conducted on these organs/tissues according to OECD guidelines. Limited scope in fixation of organs/tissues and histopathological examination was considered in the other studies (see Table 1).

Statistical analysis. For body weight, haematological, clinical chemistry parameters and organ weight data analysis of covariance was carried out using the methods of ANOVA. If a significant difference was observed between groups the mean values were compared by Dunnett's test. Where there were fewer than eight animals per group a statistical one-sided analysis was carried out using the Kruskal–Wallis *t*-test.

RESULTS

Confirmation of exposure data

The concentrations of NVP in the inhalation exposure chambers and in water (drinking water and gavage studies) as ascertained by analysis were found to be within ±3.5% of the intended concentrations in air, and ±3.7% of those in water. The stability of NVP during periods of up to 6 months was confirmed by analysis. In the case of studies of longer duration, freshly produced NVP was introduced for use at intervals of 6 months or less.

Body weight

Inhalation exposure studies. Inhalation of NVP vapour at concentrations of 5 ppm or more for 6 hr/day on 5 days/wk resulted in a concentration-related reduction in body weight gain. After exposure to 45 ppm for only 1 day, body weight was significantly less than that of controls in the case of mice and rats (studies 1 and 2). The same was true for hamsters after exposure to 45 ppm for 9 days (study 5, see Fig. 1). In response to 20 ppm, male Sprague–Dawley rats (study 11) appeared to exhibit significantly reduced body weight gain from wk 1 onwards, and as shown in Table 2, this body weight deficit persisted in the satellite group up to the time the animals were killed at 3 months. However, it should be noted that males in the 20 ppm exposed group weighed less than the control rats before the start of exposure. By contrast, similar exposure of female Sprague–Dawley rats was associated with only a slight, and not statistically significant, reduction in body weight gain (see Table 2). Because data were available for larger numbers of animals, the effects of inhalation exposure on body weight gain were more clearly seen during the first 3 months of a long-term study in Sprague–Dawley rats (Klimisch *et al.*, 1997). Notwithstanding the fact that the mean weight of male rats destined to be exposed to 10 ppm NVP was significantly lower than that of control rats before the start of exposure, there appeared, as shown in Table 3, to be a dose-related (i.e. concentration/time-related) effect on body weight gain in both sexes, with significant deficits apparent in response to 10 ppm throughout the study in males, and from day 13 onwards in females, and in response to 5 ppm from day 13 in males. As in the shorter-term rat study, the deficit in weight gain in males was higher (approximately twice) than that in females (Table 4). No effect on body weight gain was seen in Sprague–Dawley rats exposed to NVP at a concentration of 1 ppm for 3 months (study 6).

Groups of 20 male and 20 female mice exposed for up to 7 wk to 5 or 15 ppm NVP (study 4) showed no significant effect on body weight gain. The same is true for F344 rats similarly exposed to NVP (study 3).

Drinking water and gavage studies. Administration of up to 7.5 mg/kg body weight/day NVP in drinking water to rats for 3 months (study 7) had no effect on body weight; administration by gavage (study 8) had only a marginal, and non-significant, effect (body weight decrease approx. 5%) at the highest dose of 100 mg/kg body weight/day.

Clinical findings

Rats showed severe signs of toxicity during the first week of exposure to 120 ppm NVP by inhalation (study 6). The signs included irritant effects to the eyes and nose, rapid breathing, cyanosis, apathy and haematuria. Furthermore, 16 of the 20 rats died. None of the rats or mice in studies 1–4 and 6 that were exposed by inhalation to 45 ppm NVP for periods ranging from 1 to 13 wk died. However, during the first 2 wk of such exposure some animals (both rats and mice) showed signs of slight irritation and apathy. Thereafter, no signs of abnormal behaviour or toxicity were observed, indicating that the animals adapted to exposure.

No signs of toxicity were observed in Syrian hamsters exposed to 45 ppm by inhalation for 3 months (study 5) or in rats, mice or hamsters exposed by inhalation to concentrations ranging up to 20 ppm for periods of up to 12 months (studies 3, 4, 6, 9, 10 and 11).

In response to exposure to NVP in drinking water or by gavage (studies 7 and 8) no effects were seen in rats exposed to up to 75 ppm (drinking water) or 100 mg/kg/day (gavage).

Ophthalmoscopy revealed no differences between NVP-exposed animals and control animals in any of the studies.

Haematological findings and clinical chemistry

Table 5 summarizes the effects of NVP on clinical chemistry and haematological parameters in studies 3–11. Most of the effects on clinical chemistry par-

Table 6. Organ weight and histopathological findings

Study	Type of study	Species/doses	Observations
1	Inhalation 1 wk	F344 rats, 45 ppm	Liver: fatty infiltration, centrilobular single cell necrosis: 45 ppm
2	Inhalation 1 wk	$C57B_1$ mice, 45 ppm	Liver: fatty infiltration, centrilobular enlarged hepatocytes, single cell necrosis: 45 ppm
3	Inhalation 1, 3, 7 wk	F344 rats, 5, 15, 45 ppm	Liver: increased relative liver weight in females: 45 ppm (1, 3, 7 wk) centrilobular single cell necrosis, degeneration: 45 ppm (1, 3, 7 wk) foci of cellular alteration: 45 ppm (7 wk) Nasal cavity: atrophy of olfactory epithelium: 15, 45 ppm (1, 3, 7 wk)
4	Inhalation (1, 3, 7 wk)	$C57B_1$ mice 5, 15, 45 ppm	Liver: increased liver weight: 45 ppm (3, 7 wk), centrilobular enlargement of hepatocytes: 45 ppm (1, 3, 7 wk), centrilobular single cell necrosis, degeneration: 45 ppm (1 wk) Nasal cavity: atrophy of olfactory epithelium: 5, 15, 45 ppm (1, 3, 7 wk), hyperplasia of respiratory epithelium: 5 ppm (7 wk), 15, 45 ppm (1, 3, 7 wk) hyperplasia of submucosal glands: 5, 15, 45 ppm (7 wk) Trachea: epithelial proliferation, single cell necrosis: 45 ppm (1, 3, 7 wk) Lungs: bronchial epithelium slightly proliferated: 5 ppm (7 wk),15 ppm (3, 7 wk), 45 ppm (1, 3, 7 wk)
5	Inhalation 7 wk, 3 months	Syrian hamsters, 45 ppm	Liver: accumulation of glycogen (7 wk, 3 months)
6	Inhalation 3 months	Sprague–Dawley rats, 1, 5, 15, 45, 120 ppm	Most of the animals treated with 120 ppm died. Liver: increased liver weight: 15, 45 ppm, dystrophy: 120 ppm, hypertrophy of hepatocytes: 45, 120 ppm, foci of cellular alteration: 15, 45 ppm Nasal cavity: inflammation: 5, 15, 45, 120 ppm, atrophy of olfactory epithelium: 5, 15, 45, 120 ppm, hyperplasia of basal cells of respiratory and olfactory epithelium: 5, 15, 45 ppm
7	Drinking water 3 months	Wistar rats, 0.5, 1.2, 3.0, 7.5 mg/kg/day	No changes
8	Gavage 13 wk	Wistar rats, 40, 60, 100 mg/kg/d (5 days/wk)	Liver: increased liver weight: 40 mg/kg in females 60, 100 mg/kg in males and females foci of cellular alteration: 100 mg/kg
9	Inhalation 6 months	F344 rats, 10 ppm	No changes
10	Inhalation 6 months	$C57B_1$ mice, 10 ppm	No changes
11	Inhalation 3, 12 months	Sprague–Dawley rats, 5, 10, 20 ppm	Liver: increased liver weight: 20 ppm (3, 12 months), foci of cellular alteration (clear cell areas): 5 ppm (12 months: females, only) 10, 20 ppm (3, 12 months,) spongiosis hepatis: 20 ppm (12 months) Nasal cavity: inflammation: 5, 10, 20 ppm (3, 12 months), atrophy of olfactory epithelium: 5, 10, 20 ppm (3, 12 months), hyperplasia of basal cells of respiratory and olfactory epithelium: 5, 10, 20 ppm (3, 12 months), hyperplasia of submucosal glands: 5, 10, 20 ppm (12 months), adenoma: 5 ppm: one male, 20 ppm: each one animal and female (12 months)

ameters were indicative of hepatotoxicity, but a few, including decreased plasma levels of creatinine, urea and potassium, were more probably non-specific consequences of poor general condition.

After inhalation exposure the most important clinical chemistry findings are attributable to damage and/or functional impairment of the liver. In rats, the decreased plasma concentrations of total protein, albumin and globulins and the increased γ-GT activity and GSH concentration in the liver are secondary to hepatocellular changes. These changes were seen in both F344 and Sprague–Dawley rats after inhalation of 5 ppm up to 45 ppm NVP for up to 3 months and were already evident after exposure for only 1 wk. After an inhalation exposure for 7 wk or 3 months the changes in clinical chemistry parameters indicative of hepatocellular damage were not so pronounced as those seen at the beginning of the study. This indicates that animals adapted to exposure to the test compound. The range of effects on clinical chemistry parameters indicative of hepatocellular toxicity were similar in the two strains of rat exposed to NVP by the inhalation route. Oral administration of the test compound to Wistar rats by gavage or through the drinking water was also associated with hepatotoxicity, as evidenced by increased liver γ-GT (at all dose levels) and decreased plasma protein levels (at the top dose level).

In both studies in C57BL mice, inhalation of 10–45 ppm NVP caused decreases in plasma total protein, albumin and globulins and an increase in liver GSH. As in rats, these effects were most prominent at the beginning of the exposure period.

No changes in clinical chemistry parameters indicative of liver toxicity were seen in hamsters exposed to 45 ppm NVP for 3 months.

Urinalysis revealed no differences of toxicological significance in any of these studies.

Decreases in haemoglobin and various red blood cell parameters, although not on red blood cell morphology, are suggestive of an adverse effect of NVP on red blood cells, indicative of a microcytic anaemia. The increased counts of circulating neutrophils and lymphocytes in study 4 are, however, more likely to have been secondary to inflammatory changes in the nasal cavity, trachea and lungs. Similarly, the increases in circulating neutrophils and lymphocytes in studies 6 and 11 were probably secondary to the inflammatory changes in the nasal cavity.

F344 rats showed increases in haemoglobin, red blood cells and haematocrit at 45 ppm NVP for 1 or 3 wk (study 3). However, after the inhalation of 15 or 45 ppm NVP for 6 wk or longer decreases in haemoglobin, red blood cells and haematocrit were observed in F344 rats (study 3), Sprague–Dawley rats (studies 6 and 11) and C57BL mice (study 7). These findings suggest that NVP adversely affects erythropoiesis. The increases in platelet counts seen in studies 3, 4, 6, 8, 9 and 11, are probably a concomitant finding of the anaemic process. No changes in haemoglobin, red blood cells or haematocrit were seen in mice exposed to 5 ppm NVP for up to 7 wk (study 4) or to 10 ppm for 6 months (study 10) and no haematological changes were seen in hamsters (study 5) or in rats exposed through the drinking water (study 7).

Organ weights and histopathological findings. Table 6 lists exposure-related findings following exposure of rats, mice and hamsters to NVP in various doses and by various routes of administration. Target tissues were the liver (all three species) and the respiratory system (rats and mice only).

In rats and mice, fatty infiltration and centrilobular single-cell necrosis were observed in the liver after only 1 wk of exposure. In mice, additionally, centrilobular hypertrophy of hepatocytes was also seen at this time. After 7 wk or 3 months of treatment, foci of altered hepatocytes were observed in all three strains of rat after inhalation of 15 or 45 ppm NVP or gavage of 100 mg/kg body weight/day. Glycogen accumulation was observed in the liver of hamsters after inhalation of 45 ppm NVP for either 7 wk or 3 months. After exposure for 12 months, foci of altered hepatocyes were seen in rats in response to the inhalation of 5 ppm (females, only), and 10 ppm or 20 ppm (both sexes). Additionally, spongiosis hepatis, a degenerative lesion of hepatocytes, was observed in rats exposed to 20 ppm for 12 months. In most of these studies, the histopathological changes in the liver were accompanied by increased relative liver weight. In study 6, after inhalation of 120 ppm NVP, most rats died, showing dystrophy of the liver.

In the nasal cavity of rats and mice, atrophy of the olfactory epithelium was seen as early as only 1 wk of inhalation exposure to 5 ppm NVP, and in Sprague–Dawley rats, inflammation and hyperplasia of the basal cells of both the respiratory and olfactory epithelium was seen after 3 or 12 months of exposure. After 7 wk of exposure of mice to 5 ppm or more NVP and after 12 months exposure of rats to 5 ppm or more NVP, hyperplasia of submucosal glands was observed. Also, adenomas were seen in one male exposed to 5 ppm and in one male and one female exposed to 20 ppm. In mice, hyperplasia of the respiratory epithelium was observed in animals exposed to 15 or 45 ppm for 1, 3 or 7 wk and in animals exposed to 5 ppm for 7 wk. Additionally, hyperplasia of submucosal glands was seen in mice that had inhaled 5, 15 or 45 ppm NVP for 7 wk.

In mice, but not in rats, exposure-related effects were observed in the trachea and lungs. These consisted of proliferation and single-cell necrosis of the epithelium of the trachea (after 1–7 wk of exposure to 45 ppm) and slight proliferation of the epithelium of bronchi and alveoli (see Table 6, study 4).

No effects were observed in rats or mice after 6 months' inhalation of 10 ppm NVP, nor after 3 months oral exposure to NVP through the drinking water in doses up to 7.5 mg/kg body weight/day.

Exposure of rats by gavage to doses of 40–100 mg/kg body weight/day NVP on 5 days/wk for 13 wk (study 8) was associated with increased liver weight, with females being more susceptible than males. Foci of hepatocellular alteration were seen in animals given 100 mg/kg body weight/day, but not in animals given lower daily doses.

DISCUSSION

A priori, it is to be expected that monomeric structures used in the production of polymers will be found to be irritant because they may react with proteins and other tissue cell components. The finding of damage, especially to the nasal epithelium, following the inhalation of NVP was not therefore unexpected. On the other hand, the effects on haematological parameters and on the livers of animals were not anticipated until the data reported by Greim (1994) and the VCI (1992) became available.

In the interpretation of the findings reported in this paper it should be borne in mind that grooming after whole-body exposure to NVP in inhalation chambers could have led to oral exposure to the chemical.

The following paragraphs compare the effects of oral and inhalation exposure, consider the mechanisms that might be involved, and discuss the findings in relation to dose selection for a long-term inhalation study in rats.

Effects of oral exposure

In study 8 exposure of Wistar rats by gavage to 40, 60 or 100 mg/kg body weight/day on 5 days/wk for 3 months led to increased liver γ-GT at all levels in both sexes, to marginally reduced body weight gain in high-dose animals, to increased relative liver weight in all dose levels in females and in the middle- and high-dose groups in males, and to foci of hepatocellular alteration in high-dose animals. In study 7 no adverse effect on body weight was seen in response exposure through the drinking water to doses of up to 7.5 mg/kg body weight/day on 7 days/wk. Also, there were no abnormal haematological or histopathological findings. However, decreases in plasma proteins, albumin and globulins were seen in response to 7.5 mg/kg body weight/day. Thus, 3.0 mg/kg body weight/day was a NOAEL in this study.

Effects of inhalation exposure

In the nine studies involving exposure of rats (two different strains), mice and hamsters to NVP by the inhalation route, there was a reasonably consistent pattern of response in rats and mice, with adverse effects being seen on body weight gain, haematological and clinical chemistry parameters and histopathological findings in the liver and nasal cavity. In hamsters, toxic manifestations were far less prevalent. This may be because the hamster is able to reduce its exposure by going into a semi-hibernatic state. The clinical chemistry findings, which included effects on plasma proteins, enzymes and bilirubin, effects on γ-GT and GSH in liver cells, and macroscopic and histopathological changes in the liver, which included increased liver weight, fatty infiltration, centrilobular hepatocellular enlargement and foci of hepatocellular alteration, pointed to the liver being a main target for the systemic toxicity of NVP in rats and mice. In hamsters, the only effects noted after exposure to 45 ppm NVP for up to 3 months were reduced body weight gain and increased accumulation of glycogen in the liver. For rats exposed to NVP for 3 months, the NOAEL for the liver was 5 ppm and that for the nasal mucosa was 1 ppm.

Inhalation exposure to NVP at concentration levels of 15 ppm or higher for 6 wk or longer caused a mild, hypochromic, microcytic anaemia in rats of both sexes (studies 3, 4, 6 and 11). As no significant changes were observed in the morphology of red blood cells, the effect on erythrocytes appears to be due to decreased cell production rather than to increased destruction of cells. The decreases in mean corpuscular volume and mean corpuscular haemoglobin seen in studies 6 and 11 also indicate that the anaemia is probably caused by a slight disturbance of haemoglobin synthesis.

The decrease in plasma protein levels (studies 3, 4, 6, 7, 9, 10 and 11) is probably a consequence of hepatotoxicity. The changes noted were generally more pronounced shortly after the start of exposure than towards the end of studies. The regression of the decreases in plasma protein concentrations during the course of the study indicates that the finding of dysproteinaemia is not a progressive effect. In other words, it seems that the treated animals adapt to exposure.

Atrophy of the olfactory epithelium was seen in F344 rats exposed to 15 or 45 ppm NVP for 1, 3 or 7 wk (study 3) but not to 10 ppm for 6 months (study 9), and in Sprague–Dawley rats exposed to 5, 15, 45 or 120 ppm for 3 months (study 6) or to 5, 10 or 20 ppm for 3 or 12 months (study 11). Hyperplasia of basal cells of the respiratory and olfactory epithelium was also seen at these dose levels in studies 6 and 11. Also, in study 11 at 12 months there was hyperplasia of the submucosal glands and a total of three adenomas arising in the nasal epithelium. In mice, atrophy of olfactory epithelium, hyperplasia of respiratory epithelium and hyperplasia of submucosal glands were seen in response to exposure to 5 ppm or higher concentrations of NVP for up to 7 wk (study 4).

The only other histopathological effects seen in any of the three species studied were epithelial proliferation and single cell necrosis in the trachea in response to the inhalation of 45 ppm NVP for 7 wk in mice (study 4), and slight proliferation of bronchial epithelium in response to 5, 15 or 45 ppm for up to 7 wk in the same study.

Possible mechanisms

The fact that no changes were seen in the upper or lower respiratory tracts of rats exposed to NVP by the oral route (studies 7 and 8) suggests that the effects on these tissues seen in the studies involving exposure by the inhalation route depend on their direct local contact with absorbed NVP.

The fact that platelet counts were increased in rats of both sexes exposed by gavage to 60 or 100 mg/kg body weight/day for 3 months (study 8) is indicative of an effect, either of NVP itself or of a metabolite of NVP, on the bone marrow in this study. However, it seems clear that oral exposure to NVP is far less productive of haematological effects than inhalation exposure to NVP. Choi and Simone (1973) reported an inverse relationship between platelet counts and haematocrit in rats suffering from iron-deficiency anaemia. It is possible therefore that the effect of NVP on platelet counts is secondary to an effect of NVP on some aspect of erythropoiesis.

Although the clinical chemistry findings are consistent with there being weak hepatotoxicity in the two studies involving oral exposure (studies 7 and 8), it is clear that inhalation exposure led to far more marked hepatotoxicity. It is not known whether the various effects on the liver are attributable to NVP itself or to one or more metabolites of NVP. However, the fact that no evidence of toxicity was seen in tissues such as the kidney is consistent with the probability that either NVP itself, or more likely a short-lived metabolite of NVP, produced in the liver may be responsible for these effects. Again, it is not known whether the effects on haematological parameters are due to NVP itself or to one or more metabolites of NVP.

One would have expected that adverse effects on the liver would have been more marked after oral exposure than after inhalation exposure to NVP. The fact that the opposite appears to be the case is puzzling in the absence of precise comparative pharmacokinetic data. The possibility that exposure of the liver to inhaled NVP delivered through the hepatic artery is more hepatotoxic than NVP reaching the liver through the portal vein after oral exposure cannot be ruled out. Nor can partial detoxification of NVP in the gastrointestinal tract, or activation of NVP in the lungs, be excluded.

Dose selection for a long-term chronic toxicity/carcinogenicity inhalation study in rats

At the time the long-term inhalation study in rats (Klimisch *et al.*, 1997) was planned, the only findings available for consideration were from studies 1, 3, 6 and 9. Study 11, although reported in the present paper, was conducted as part of the long-term study. Experience in studies 1, 3, 6 and 9 suggested that 5 ppm would probably be a NOAEL for hepatotoxicity (see Table 6). On the other hand, it was clear that exposure to this concentration of NVP would give rise to inflammatory changes in the nasal cavity (see Table 6). It was assumed that these latter changes were indicative of no more than local irritation and that they could be ignored in a study aimed at defining a NOAEL in respect of the systemic toxicity of NVP.

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